Effects of Lysine Deficiencies on Plasma Levels of Thyroid Hormones, Insulin-like Growth Factors I and II, Liver and Body Weights, and Feed Intake in Growing Chickens

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ABSTRACT Adequate (1.10%) and deficient (0.88, 0.66, and 0.53%) levels of Lys were fed to broiler chicks from 9 to 23 d of age. Groups fed the control diet (1.10% Lys) were also pair-fed daily with each deficient group. Compared with the free-fed control, graded decreases in feed intake occurred as the deficiency worsened, and these were significantly different with 0.66 and 0.53% Lys. Growth decreased significantly with each deficient level of Lys compared with the free-fed control and was always significantly lower than in the pair-fed control groups in each set. Plasma triiodothyronine (T_3) was elevated in chicks fed 0.88 and 0.66% lysine but not with 0.53% when compared with the full-fed control treatment. However, in deficient chicks receiving 0.66 and 0.53% Lys, T_3 levels were significantly higher compared with their pair-fed controls. Plasma T_4 was not significantly different between any treatments. Liver weights decreased significantly at each level of Lys deficiency, but most of the differences disappeared when expressed relative to body weight. Plasma insulin-like growth factor (IGF)-I decreased significantly with the most severe Lys deficiency. However, it decreased to a similar degree in the pair-fed controls, showing that this effect was primarily due to the lower feed intake. Plasma IGF-II levels did not differ between any treatments. No correlations were evident between thyroid hormones and IGF-I or IGF-II values. We concluded that the primary effect of Lys deficiency was an elevation in plasma T_3 levels without accompanying changes in plasma T_4. No effect of the Lys deficiency per se on plasma IGF-I and IGF-II and liver weights relative to body weights was found.

(*Key words*: broiler chick, insulin-like growth factor, liver weight, lysine deficiency, thyroid hormone)

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INTRODUCTION

In standard formulations of broiler diets in the United States and many parts of the world, Lys is frequently a limiting amino acid, and it is supplemented into such diets. Although the negative effects of a Lys deficiency on growth and feed intake are well documented, little is known about their effects on the avian endocrine system.

A study of the effects of deficiencies of essential amino acids (EAA) on thyroid function has shown that dietary deficits of individual EAA have differing effects on blood levels of triiodothyronine (T_3) and thyroxine (T_4) in growing chickens (Carew et al., 1997). In this 14-d study, they reported that Lys at 60% of the recommended (NRC, 1994) level of 1.1% causes an elevation in plasma T_3 compared with pair-fed controls but no change when compared with free-fed controls. The changes were just the opposite with T_4, which were lower in Lys-deficient chicks compared with the free-fed control but not when compared with pair-fed controls. Earlier, Elkin et al. (1980) in a 14-d chick study with Lys at 53% of the recommended level had reported similar results. They observed no effect on serum T_3 levels compared with the free-fed control, whereas deficiency caused a decrease in serum T_4. However, they did not have pair-fed controls for comparison. May (1979) observed no effect on serum T_3 or T_4 when Lys was fed at 50% of the requirement for 2 d. The short duration of this study probably limited any response. In rats, a Lys deficiency was reported to cause elevations in total serum T_3 and T_4 but not in the free T_4 index (Cree and Schalch, 1985).

We found no studies on the relationship between Lys deficiency and circulating levels of insulin-like growth factors (IGF)-I and II in chickens. For growing rats, Bolze et al. (1985) reported a decrease in somatomedin (early name for IGF) with a Lys deficiency and, based on results with pair-fed controls, concluded that the effect was not

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Abbreviation Key: EAA = essential amino acids; IGF = insulin-like growth factor; T_3 = triiodothyronine; T_4 = thyroxine.

TABLE 1. Composition of broiler starter diet[1]

Ingredient	%
Yellow corn meal	51.07
Soybean meal (dehulled)	35.40
Soybean oil	4.00
Poultry blend meal	3.00
Alfalfa meal	1.00
Distillers dried grains	2.00
Dicalcium phosphate	1.30
Limestone	1.10
Salt	0.40
Vitamin mix[2]	0.50
Trace mineral mix[3]	0.10
DL-Methionine	0.13

[1]Contained (by calculation) 24.4% protein and 3,148 kcal of ME/kg. In percentages, Arg, 1.60; Cys, 0.47; Gly + Ser, 3.02; His, 0.63; Ile, 1.00; Lys, 1.31; Met, 0.51; Phe, 1.15; Thr, 0.90; Trp, 0.28; Tyr, 0.94; Val, 1.13.

[2]Supplied (mg/kg of diet): riboflavin, 4.4; D-pantothenic acid Ca-salt, 8.8; niacin, 20; choline, 322; menadione, 2; vitamin E, 11; vitamin B_{12}, 0.01; ethoxyquin, 33; glucose, 4,569.2. In IU/kg diet: vitamin A, 5,200; vitamin D_3, 1,000.

[3]Supplied (mg/kg of diet): Fe, 40, as $FeSO_4 \cdot 7H_2O$; Mn, 75, as $MnSO_4 \cdot H_2O$; Zn, 60, as ZnO; Cu, 4, as $CuSO_4 \cdot 5H_2O$; I, 0.86, as KIO_3; Se, 0.10, as Na_2SeO_3; Ca, 136, as $CaCO_3$.

TABLE 2. Composition of purified amino acid diet[1]

Ingredient	%
Sucrose	59.364
Amino acid mix[2]	23.620
Mineral mix[3]	8.270
Soybean oil	5.000
Cellulose (Solka-Floc)[4]	3.000
Vitamin mix[5]	0.500
Choline chloride (70%)	0.233
Ethoxyquin[6]	0.013

[1]Equivalent to 24% protein.

[2]Supplied (L-isomers except Met and Gly) in percentage of diet: Trp, 0.22; His $HCl \cdot H_2O$, 0.41; Tyr, 0.63; Phe, 0.68; Met, 0.55; Cys, 0.35; Thr, 0.85; Leu, 1.20; Ile, 0.80; Val, 0.82; Gly, 1.60; Pro, 1.00; Lys HCl, 1.40; Arg HCl, 1.33; Glu, 12.00.

[3]Supplied (g/kg of diet): $CaHPO_4$, 27; $CaCO_3$, 11; $NaHCO_3$, 15; KH_2PO_4, 9; NaCl, 8.8; $MnSO_4 \cdot 7H_2O$, 0.70; $FeSO_4 \cdot 7H_2O$, 0.5; $MgSO_4 \cdot 7H_2O$, 3.5; KIO_3, 0.0025; $CuSO_4 \cdot 5H_2O$, 0.03; H_3BO_3, 0.009; ZnO, 0.105; $CoCl_2 \cdot 6H_2O$, 0.002; $NaMoO_4 \cdot 2H_2O$, 0.009; Na_2SeO_3, 0.0002; sucrose, 7.045.

[4]Brown Co., Berlin, NH.

[5]Supplied (mg/kg of diet): riboflavin, 15; D-pantothenic acid Ca-salt, 50; niacin, 100; pyridoxine HCl, 15; thiamin HCl, 15; folacin, 6; biotin, 0.5; vitamin B_{12}, 0.05; menadione Na bisulfite (63 to 75%), 5. In IU/kg diet: vitamin A, 9,750; cholecalciferol, 4,800; DL-α-tocopheryl acetate, 50; glucose, 4,569.2.

[6]1,2′-dihydro-6-ethoxy-2,2,4-trimethylquinoline (Solutia, St. Louis, MO).

specifically due to the Lys deficiency but was a consequence of the deficit of calories and protein. Cree and Schalch (1985), using a Lys-deficient diet based on wheat gluten, found that somatomedin was lower in age-matched rats but not in weight-matched rats, again suggesting that level of feed intake and not the Lys per se accounted for the effect. To the contrary, Takenaka et al. (2000) found that Lys-deficient growing rats had significantly lower plasma IGF-I levels compared with free-fed or pair-fed controls. However, IGF binding protein-I levels in the plasma or liver did not differ from either control.

Because earlier research in chickens with thyroid hormones was done using only severe Lys deficiencies and no results are available concerning IGF-I and IGF-II, the purpose of the present study was to determine the effect of graded Lys deficiencies from borderline to severe on these 2 groups of hormones.

MATERIALS AND METHODS

Bird Management

The experimental protocol was approved by the Institutional Animal Care and Use Committee. During the pre-experimental period of 1 to 9 d of age, 175 male broiler chicks[2] were housed in electrically heated Petersime starter battery brooders with raised wire floors and internal lighting. They were fed a control diet complete in all nutrients including essential amino acids (Table 1). At 9 d of age chicks were evenly distributed into 21 pens of 7 each (147 total chicks) with similar average weights (178 ± 1 g) and weight ranges; high and low weight extremes were eliminated. Triplicate groups of chicks were used per treatment. The experiment lasted 2 wk from 9 to 23 d of age. The treatments were a control (1.10%) and 3 deficient levels of Lys (0.88, 0.66, and 0.53%). Groups of control chicks were pair-fed with the deficient chicks by matching a pen of chicks fed the control diet with one of the deficient pens, and feeding it once daily, at 0800 h, the same amount of feed that the deficient chicks had eaten the previous day. This was done to account for the effect of differences in feed intake between the control group given free access and the deficient groups. Brooder temperatures were as follows: wk 1, 35°C; wk 2, 32°C; and wk 3, 29°C. Room temperature was maintained between 23 and 26°C. Water was given freely and a cycle of 16L:8D was used.

[2]Hubbard Farms, Walpole, NH.

Diets and Experimental Design

The nutritionally complete control diet used during the 14-d experimental period consisted of a 40/60 mixture of a broiler starter diet (Table 1) and a purified amino acid diet (Table 2) slightly modified from Scott et al. (1982). It contained 24% protein, 3.4 kcal of ME/g, and 1.1% Lys supplied mainly by highly digestible pure Lys. The Lys-deficient diets (0.88, 0.66, and 0.53% Lys) were formulated by replacing appropriate amounts of Lys in the purified portion of the mixed diet with sucrose to maintain isocaloric diets. All other amino acids remained the same. A randomized complete block design was used.

Blood Samples, Hormone Assays, and Statistics

At 23 d, blood was drawn from each chick by side heart puncture between 1000 and 1300 h and was placed into

TABLE 3. Feed intake, growth rate and liver weights of male broilers deficient in lysine[1]

Treatment	Dietary Lys (%)	Feed intake (g)	Weight gain (g)	Feed efficiency (gain/feed)	Liver weight	
					True (g)	Relative (g/kg of BW)
Control	1.10 Free-fed	936[a]	672[a]	0.72[a]	24.3[a]	28.3[ab]
Deficient	0.88 Free-fed	863[a]	540[c]	0.62[c]	21.5[b]	30.1[a]
Control	1.10 Pair-fed	863[a]	615[b]	0.71[a]	25.1[a]	30.7[a]
Deficient	0.66 Free-fed	604[b]	304[e]	0.50[d]	13.9[d]	28.4[ab]
Control	1.10 Pair-fed	634[b]	424[d]	0.67[b]	17.8[c]	29.8[a]
Deficient	0.53 Free-fed	448[c]	177[f]	0.39[e]	8.9[e]	25.8[b]
Control	1.10 Pair-fed	468[c]	286[e]	0.61[c]	13.6[d]	28.5[ab]
SEM		40.8	28.4	0.02	3.37	3.48

[a–f]Means within a column lacking a common superscript differ from each other ($P < 0.05$).

[1]The experimental period was from 9 to 23 d of age. Feed and weight gain data represent triplicate groups of 7 chicks each. Liver data represent 12 observations per treatment.

heparinized tubes. The tubes were centrifuged at 1,800 × *g* for 15 min to obtain plasma, which was frozen for later analysis. Plasma total T_3 and T_4 levels were analyzed by RIA,[3] which had been validated as described previously (Carew et al., 1997, 2003). IGF-I and IGF-II were analyzed by homologous RIA (McMurtry et al., 1994, 1998).

Data were analyzed using the GLM analysis of variance with $P < 0.05$ considered significant (SAS Institute, 1990). Means were compared with Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

When dietary Lys was fed at levels below 1.10% (0.88, 0.66, and 0.53%), feed intake was not significantly affected at 0.88% but decreased significantly at 0.66 and 0.53% Lys, the latter having the most severe effect (Table 3). Feed intake of pair-fed controls was close to expectation. Weight gain significantly decreased in a graduated manner with all 3 deficient levels of Lys (Table 3) compared with the free-fed control. In all cases, pair-fed controls weighed significantly more than their matched groups. The overall result of these changes in feed intake and weight gain was that weight gain to feed intake ratios decreased significantly at every level of Lys deficiency. These results are different from those reported earlier with a deficiency of Met, another major limiting EAA. With a Met deficiency, the level of dietary Met required to produce maximum efficiency has been shown to be slightly higher than that needed to produce maximum growth (Slinger et al., 1953; Nelson et al. 1960; Carew and Hill, 1961). Our results with a Lys deficiency also differed from a Met deficiency in that a marginal deficiency of Met actually causes a significant increase in feed intake that returns to control level when sufficient Met is fed (Slinger et al., 1953; Nelson et al., 1960; Carew and Hill, 1961); no such effect was observed with Lys in the present study.

The markedly poorer growth of chicks deficient in Lys compared with their matched, pair-fed controls (Table 3) strongly suggests that major changes in body composition or metabolism had occurred. This could result from an increase in fat synthesis at the expense of body protein in the deficient chicks or decreased energetic efficiency with increased heat loss. That the latter is not the case is suggested by the studies of Peters (1963), who reported that a normal linear relationship existed between metabolizable energy intake and tissue energy gains over a wide range of food intakes in young growing chicks deficient in Lys. However, other detailed studies of energy metabolism in Lys-deficient chickens have not been reported. In studies with a Met deficiency, shifts in body composition from protein to fat with no change in apparent heat production have been observed with a mild deficiency (Carew and Hill, 1961), whereas a very severe deficiency caused increased heat production (Sekiz et al., 1975). Thus the situation with regard to energy metabolism with deficiencies of Lys or Met seems quite different. This demonstrates that no single mechanism with regard to energetic efficiency can be applied to deficiencies of all EAA.

Liver weight is often an index of protein and other nutrient deficiencies (Velu et al., 1971). In the present study, true liver weights declined significantly at each step of the Lys deficiency compared with the free-fed control and in each case were significantly smaller compared with the matched, pair-fed controls (Table 3). However, when expressed relative to body weight, most of these differences disappeared, and the only significant difference was that livers of chicks fed 0.53% Lys were smaller than those fed 0.88%. There were no differences between deficient chicks and their pair-fed controls. This lack of difference in relative liver weights between deficient chicks and pair-fed controls suggests that the Lys deficiency per se had a minimal effect on liver growth, and any differences could be explained by the smaller body size of deficient chicks, which resulted from the smaller feed intake. Thus the significant difference in relative liver weight between chicks fed 0.88 and 0.53% Lys is mostly explained by the slower growth or feed intake as demonstrated by the lack of difference within each

[3]Clinical Assays, Cambridge, MA.

TABLE 4. Blood plasma levels of thyroid hormones and insulin-like growth factors in male broilers deficient in lysine[1]

		Thyroid hormones		Insulin-like growth factors	
Treatment	Dietary Lys (%)	T_3 (ng/mL)	T_4 (μg/dL)	IGF-I (ng/mL)	IGF-II (ng/mL)
Control	1.10 Free-fed	2.65[b]	3.21[a]	54.2[a]	43.8[a]
Deficient	0.88 Free-fed	2.95[a]	3.12[a]	45.1[ab]	37.8[a]
Control	1.10 Pair-fed	2.83[ab]	3.13[a]	50.2[a]	40.9[a]
Deficient	0.66 Free-fed	3.04[a]	3.16[a]	41.5[ab]	43.4[a]
Control	1.10 Pair-fed	2.29[c]	3.12[a]	37.0[b]	38.7[a]
Deficient	0.53 Free-fed	2.66[b]	3.12[a]	35.3[b]	44.7[a]
Control	1.10 Pair-fed	2.21[c]	3.07[a]	35.8[b]	36.8[a]
SEM		0.32	0.48	16.5	14.4

[a–c]Means within a column lacking a common superscript differ ($P < 0.05$).

[1]The experimental period was from 9 to 23 d of age. Each value is the average of 14 to 15 samples taken at 22 d of age.

treatment with their pair-fed control. Changes in liver growth are different with a Met deficiency (Carew et al., 2003). When graded deficiencies of Met were fed to growing broiler chicks, they reported that liver weight relative to body weight increased. The difference may reflect the recognized role of Met in hepatic fat metabolism compared with Lys due to the need for Met in methylation processes required to properly metabolize fat. As a consequence, fat accumulation probably occurred in the livers of Met-deficient chicks but not in Lys-deficient chicks.

Plasma T_3 increased significantly in chicks fed 0.88 and 0.66% Lys when compared with the free-fed control but returned to the control level with the most severe deficiency of 0.53% (Table 4). However, when properly compared with pair-fed controls, T_3 was elevated in chicks fed the 2 diets most deficient in Lys (0.66 and 0.53%) but not with 0.88% Lys. For the severe deficiency (0.53%), this finding agrees with earlier results (Carew et al., 1997) in which only this level of deficiency was studied. Control chicks pair-fed with chicks fed the 2 lowest levels of Lys had plasma T_3 levels significantly less than the free-fed control (Table 4). It is well established that fasting as well as marked, but not mild, reductions in feed intake will cause reductions in plasma T_3 in chicks (May, 1978; Alster and Carew, 1984; Keagy et al., 1987). The results in Table 4 confirm these observations. We interpret these results to show that the normal decline in plasma T_3 that occurs concomitant with reductions in feed intake is prevented in Lys-deficient chicks, which apparently operates through a metabolic change in the deficient chicks that increases the level of circulating T_3. This was even apparent with the mildest Lys deficiency in which plasma T_3 was significantly higher than in the free-fed control, although this was somewhat confounded by the fact that it was not different from the pair-fed control. Apparently, the mechanism that depresses blood levels of T_3 in normal chicks restrict fed is altered by the Lys deficiency.

The biochemical mechanisms for the observed changes in T_3 are not known. However, we interpret our results to mean that the Lys deficiency increases the production or release of T_3 into the blood or inhibits its normal removal compared with control chicks consuming the same amount of feed. This mechanism may operate through inhibited synthesis of a key protein involved in the metabolism or turnover of T_3 due to lack of sufficient Lys for polypeptide synthesis. Similar studies with protein-deficient chicks and rats give clues to possible mechanisms. These studies suggest that elevated blood T_3 may be a consequence of increased secretion rate and activity of the thyroid gland (March et al., 1964; Tulp et al., 1979), slower clearance of T_3 from the blood (Hutchins and Newcomber, 1966), or alterations in plasma-binding capacity of the blood and changes in receptor binding or affinity (Refetoff et al., 1970; Smallridge et al., 1982; Rouaze-Romet et al., 1992), among others. Increased conversion of T_4 to T_3 due to increased hepatic or renal 5′-deiodinase activity is also a possible mechanism, but this does not occur in protein-deficient chicks (Weyland, 1993) or rats (Smallridge et al., 1982). However, in the absence of direct data with a Lys deficiency and in view of the well-known interaction of Lys with Arg (Leeson and Summers, 2001), other mechanisms of an unknown nature may be involved. Nevertheless, based on the T_3 data, it is certain that a Lys deficiency alters normal thyroid hormone metabolism.

When the most deficient level of 0.53% Lys was fed, the concentration of plasma T_3 was identical to that in the free-fed control group. This finding agrees with results reported elsewhere (Carew et al., 1997; Elkin et al., 1980), but the comparison may be meaningless because plasma T_3 is actually significantly less with this degree of restricted feed intake as shown by the pair-fed control, and the Lys deficiency raises it. Thus, it appears only coincidental that T_3 in the 0.53% Lys and the free-fed control group are almost identical, which shows the importance of having pair-fed controls in studies such as these.

No significant differences were found in plasma T_4 values among any of the treatments (Table 4). Plasma T_4 was not affected by the Lys deficiency or the restriction in food intake in the pair-fed control groups. This lack

of difference in plasma T_4 between Lys-deficient chicks and their pair-fed controls agrees with earlier results (Carew et al., 1997). However, the comparison with the free-fed control does not because they had earlier found significantly lower levels of T_4 in the Lys-deficient chicks. We have no explanation for this difference.

Compared with the free-fed control group, the plasma IGF-I level was significantly lower only with the most severe Lys deficiency. However, plasma IGF-I in the corresponding control group pair-fed to this deficient group decreased to the same degree. This result strongly suggests that the decrease in IGF-I was caused by a lack of food and not by the Lys deficiency itself. The control group pair-fed to 0.66% Lys also showed a significant decrease in plasma IGF-I. That restricted feed intake would decrease plasma IGF-I levels in chicks agrees with data of others (Kim et al., 1991; Kita et al., 1996; McMurtry et al., 1997; Beccavin et al., 2001), who reported decreases in blood IGF-I during fasting. Our data further suggest that, in addition to fasting, moderate feed restriction will also depress plasma IGF-I. Our results are also in agreement with observations with rats that the depressive effect of a Lys deficiency on blood IGF-I is due entirely to the restricted level of feed intake and not to the Lys deficiency per se (Bolze et al., 1985; Cree and Schalch, 1985).

There were no significant differences in plasma IGF-II values among any of the treatments (Table 4). IGF-II has been reported to decrease (Beccavin et al., 2001) or increase (McMurtry et al., 1998) during feed withdrawal. Our results showed no difference in either direction. We had previously reported similar negative results with restricted feeding using a similar experimental design (Carew et al., 2003), except that plasma IGF-II was elevated with the most severe degree of restriction. Such wide variation among studies of IGF-II values shows that the effect of feed restriction requires further study.

Interactions between circulating levels of thyroid hormones and IGF-I have been studied in chicks. It has been shown that elevated plasma T_3 is inversely correlated with plasma growth hormone levels in chickens (see reviews: Scanes, 1987; Harvey et al., 1991) and that plasma growth hormone and IGF-I are positively correlated. Nevertheless, research also shows that changes in IGF-I are not related to thyroidal inhibition of growth hormone (Lauterio and Scanes, 1988), and injection of chicks with T_3 or T_4 has no effect on levels of plasma IGF-I (Lazarus and Scanes, 1988). In the present study, changes in circulating levels of T_3 were not correlated with IGF-I levels (Pearson's correlation, $P > 0.05$). Also, because there were no significant differences between any T_4 values, no relationship between T_4 and IGF-I was apparent. Thus there is no evidence in this study of a relationship between changes in plasma T_3 or T_4 level and circulating levels of IGF-I.

Interactions between blood levels of thyroid hormones and IGF-II in chickens have not been reported. The relevance of IGF-II compared with IGF-I is open to question as no unique IGF-II receptor has been found in birds (McMurtry, 1998). Earlier we had reported a possible inverse relationship between plasma T_3 and IGF-II in restriction-fed chicks (Carew et al., 2003). In the present study, because there were no significant differences in plasma IGF-II values between any treatments, there was no apparent relationship between plasma T_3, plasma T_4 and IGF-II.

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